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Supplemental Information

cMyc Regulates the Size of the Premigratory Neural Crest Stem Cell Pool

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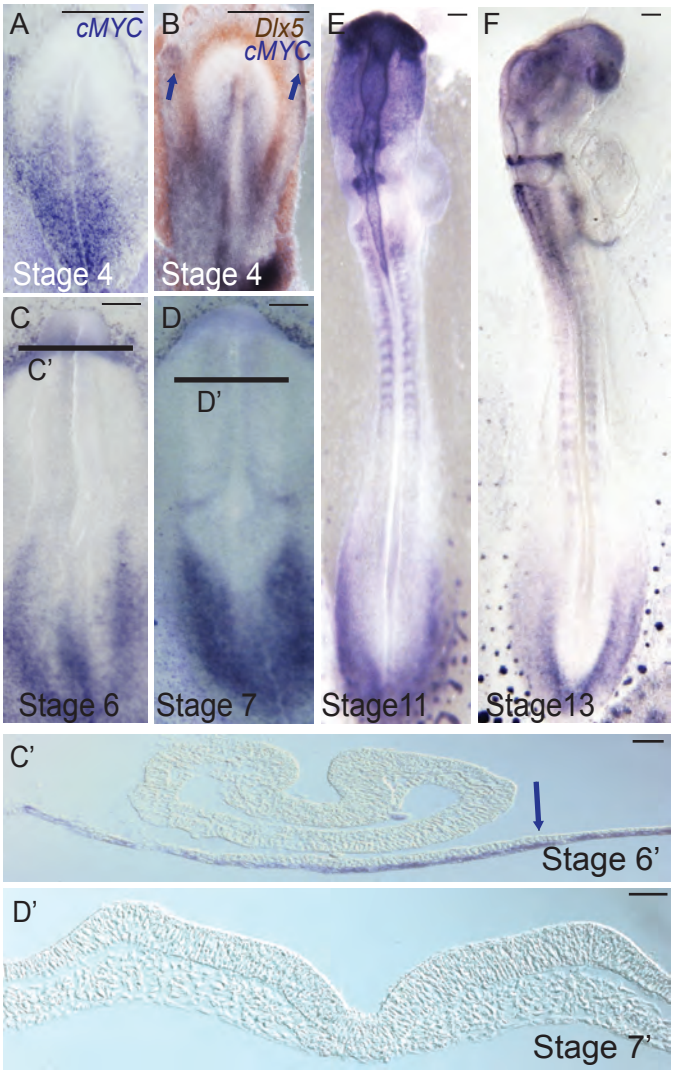
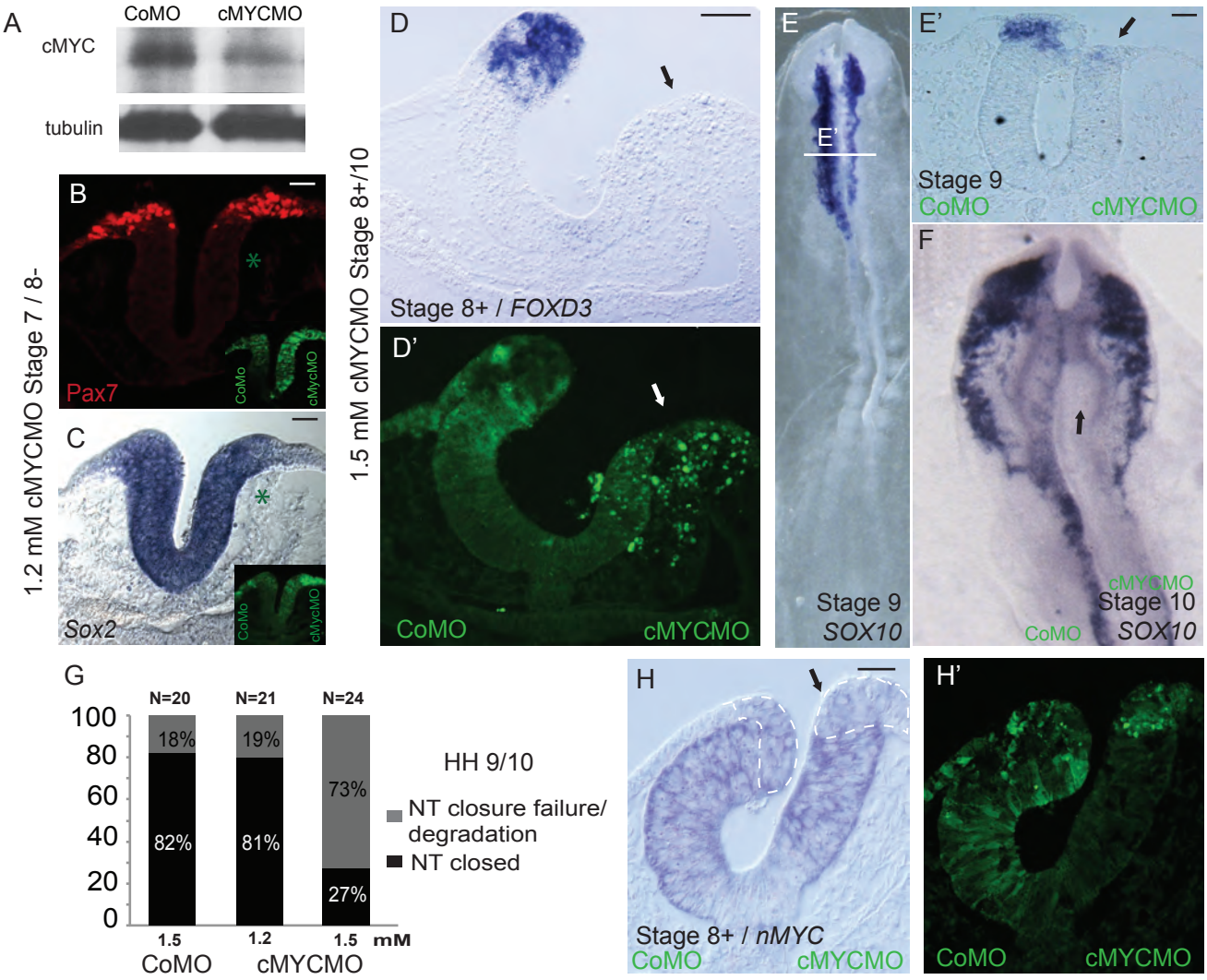
Supp fig. 1


A
cMYC
Stage 4
B
Dlx5
cMYC
Stage 4
E
F
C
C'
Stage 6
D
D'
Stage 7
Stage11
Stage13
C'
Stage 6'
D'
Stage 7'

Supplemental Figure 1. *cMyc* Is Not Expressed At The Neural Plate Border, Related to Figure 1.

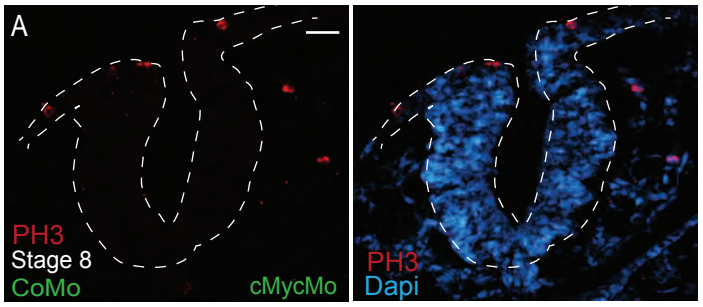
(A-B) *cMyc* RNA is not expressed at the neural plate border (marked with *Dlx5*) but rather more lateral to it as depicted with the blue arrows **(C-D, D')** and is absent from the neural folds at HH stages 6 and 7. **(C')** The anterior expression is of endodermal origin. **(E-F)** cMyc expression extends far laterally in the migrating cranial neural crest and is also emerging in the premigratory / early migrating cells at vagal and trunk levels at stages 11 and 13. Expression is also seen in blood islands in the extra-embryonic tissue. Scale bar for whole embryos 150μm; sections 20μm.

Supp fig. 2


A
CoMO
cMYCMO
cMYC
tubulin
B
Pax7
CoMo
cMycMO
C
Sox2
CoMo
cMycMO
1.2 mM cMYCMO Stage 7 / 8-
D
Stage 8+ / FOXD3
D'
CoMO
cMYCMO
1.5 mM cMYCMO Stage 8+/10
E
E'
Stage 9
SOX10
E'
Stage 9
CoMO
cMYCMO
F
cMYCMO
Stage 10
CoMO
SOX10
G
N=20
N=21
N=24
100
80
60
40
20
0
18%
82%
19%
81%
73%
27%
1.5
1.2
1.5
mM
CoMO
cMYCMO
HH 9/10
NT closure failure/ degradation
NT closed
H
Stage 8+ / nMYC
CoMO
cMYCMO
H'
CoMO
cMYCMO

Supplemental Figure 2. cMyc Morpholino Is Specific And Functions In A Dose Dependent Fashion, Related to Figure 2 (A) Western blot showing reduced levels of cMyc expression in embryonic head lysates after 1.2mM cMycMO as compared to control morpholino lysate. **(B, C)** cMyc morpholino does not affect PAX7 immunopositive neural crest cells during induction at the neural plate border or *Sox2* positive neural stem cells prior to cMyc expression at HH stage 7 (n=5/5). **(D-E, E',D')** Higher dose (1.5mM) of cMyc morpholino causes a more severe phenotype with intensive degradation of the dorsal neural tube at stages 8-9 (D') visualized with the FITC labeled morpholino. Note that the chromogenic *in situ* staining masks the FITC labeled CoMo in the control side of the dorsal neural tube. **(F-G)** At stage 9-10, degradation causes neural tube (NT) closure failure (black arrow) seen in 73% of the cMyc deficient embryos injected with 1.5mM cMycMO as compared to 1.2mM cMycMO levels that predominantly have closed neural tubes comparable to control embryos. **(H)** *cMyc* knockdown is not compensated by increased *nMyc* expression levels in the premigratory neural crest cells (n=7/7). Scale bar 20μm.

Supp fig. 3



C

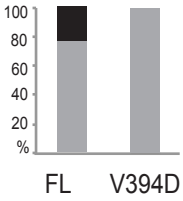
cMyc binding domain I

Miz1 chicken	NESGSTDSQENSGETRLLRSGTYSDRTESKAYGSVTHKC	X conserved
Miz1 mouse	EESAGTDSQELGMEGQNLRSGTYGDRTESKAYGSIIHKC	X conserved / similar AA switch
Miz1 frog	-E-LATDSD---SQHLGSGATPYPDRNESRPYGSTTHKC	X not conserved
Miz1 human	NESAGTDSQELGSEARGLRSGTYGDRTESKAYGSVIHKC	

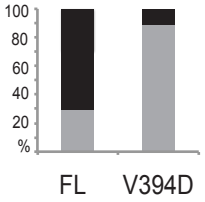
cMyc binding domain II

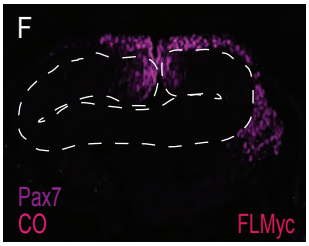
Miz1 chicken	KAGMKILEPEDG----SELNIVTVASDDMVTLATEALAATAVTQLTVVPVAAAVTADETEALKAEITKAVKQVQEADPNTQIL
Miz1 mouse	KAGIKILEPEDG----GEVSVVTV--DDMVTLATEALAATAVTQLTVVPVAAAVTADETEVLKAEISKAVKQVQEEDPNTHIL
Miz1 frog	KAGMKVLRPEDGELDEDEVNIVTVASDEMVTLTTEAIAATAVTELTVVPVTTSVAADETEALKAEISKAVKQVQEADPNTQIL
Miz1 human	KAGIKILEPEDG----SEVSVVTV--DDMVTLATEALAATAVTQLTVVPVAAAVTADETEVLKAEISKAVKQVQEEDPNTHIL

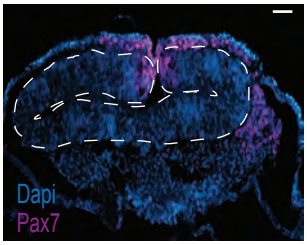
D Prolonged emigration

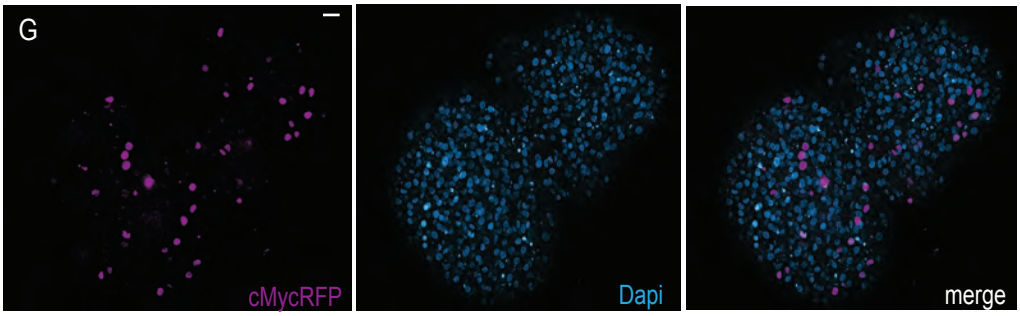


E Neural tube closure failure/ degradation









Supplemental Figure 3. Related to figures 4,5 and 6. (A) Antibody against Phospo Histone 3 shows proliferative cells in the neural tube. **(B)** In situ hybridization shows lower levels of p27 mRNA in the neural crest cell domain (circled) than in the ventral neural tube. **(C)** The two well-characterized cMyc binding domains of the Miz1 (ZBT17) protein are highly conserved among species from human to frog, which trongly suggest that the chcken Miz1 can bind to the human cMyc protein construct. The cMyc binding domain I corresponds to the area (AA 269-308 in Peukert et al., 1997) immediately before the first zinc finger; and the second domain (AA 641-715 in Peukert et al., 1997) is located in between the last (12^{th}) zinc finger and the zinc finger –like domain. The conserved areas are highlighted in yellow. The blue letters indicate conservation with a switch into another amino acid of the same functional subgroup. The sequences correspond to: Miz1_Gga GI: 171919772, Miz1_Mm GI: 443609492, Miz1_Xtr GI: 54261587 and Miz1_Hsa GI: 62906906. **(D)** FL cMyc overexpression causes prolonged emigration of newly produced neural crest cells from the dorsal neural tube in about 24% of all embryos roughly equivalent to half of the embryos that show a phenotype of increased neural crest cells in Fig. 6C) as compared to the contralateral side electroporated with a control plasmid. This is not seen in embryos overexpressing V394DMyc. **(E)** 70% of embryos that overexpress (2µg/µl) FL cMyc have neural tube closure defects compared to 11% of the V394DMyc overexpressing embryos, further showing that the Miz1 binding mutant does not cause a phenotype in the neural crest cells (FLMyc n=25; V394DMyc n=27 for both D and E). **(F)** Electroporation of a lower dosage (0.5 µg/µl) of cMyc FL is sufficient to increase the neural crest cell pool size as demonstrated by more PAX7 immunopositive cells migrating further laterally (n=4/5). **(G)** Transfection efficiency to chick crestospheres was 10-20% per sphere. Scale bar 20 µm.